

CLAIMS

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1. A polynucleotide encoding a glutathione transferase (GST) subunit, which polynucleotide comprises a coding sequence capable of hybridising selectively to the coding sequence of SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15 or 17 or to the complement of one of those sequences.
 2. A polynucleotide of claim 1 which is a DNA sequence.
 - a 3. A polynucleotide according to claim 1 ~~or 2~~ wherein the coding sequence encodes the amino acid sequence of SEQ ID No. 2, 4, 6, 8, 10, 12, 14, 16 or 18.
 - a 4. A polynucleotide according to ~~any one of the preceding claims~~ ^{Claim 1} which comprises the coding sequence of SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15 or 17 or a fragment thereof.
 5. A polypeptide which is a GST subunit and comprises the amino acid sequence of SEQ ID No. 2, 4, 6, 8, 10, 12, 14, 16 or 18 or a sequence substantially homologous thereto, or a fragment of either said sequence.
 6. A polypeptide according to claim 5 encoded by the coding sequence of a polynucleotide according to any one of claims 1 to 4.
 - a 7. A dimeric protein comprising two GST subunits, wherein at least one subunit is a polypeptide according to claim 5 ~~or 6~~.
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C2 > a 8. A chimeric gene comprising a polynucleotide according to ~~any one of claims 1 to 4~~ ^{Claim 1} operably linked to regulatory sequences that allow expression of the coding sequence in a host cell.

9. A chimeric gene according to claim 7 wherein the regulatory sequences allow expression of the coding sequence in a plant cell.
10. A vector comprising a polynucleotide according to ~~any one of claims 1 to 4~~ ^{or a chimeric gene according to claim 8 or 9.}
11. A vector according to claim 10 which is an expression vector.
12. A cell transformed or transfected with a vector according to claim 10 ~~or 11.~~
13. A cell according to claim 12 which is a prokaryotic cell or a plant cell.
14. A cell having, integrated into its genome, a chimeric gene according to claim 8 ~~or 9.~~
15. A cell according to claim 14 which is a plant cell, ~~wherein the chimeric gene is a chimeric gene according to claim 9.~~
16. A cell according to ^{claim 12} ~~any one of claims 12 to 15~~ further comprising one or more further polynucleotide sequences coding for a GST subunit, operably linked to regulatory elements that allow expression of the subunit in the cell.
17. A process for the production of a polypeptide ~~according to claim 5 or 6,~~ which process comprises:
- (a) cultivating a cell according to ^{claim 12} ~~any one of claims 12 to 15~~ under conditions that allow the expression of the polypeptide; and
- (b) recovering the expressed polypeptide.

18. A process for the production of a dimeric protein ~~according to claim 7~~, which process comprises:

(a) cultivating a cell according to any one of claims 12 to 16 under conditions that allow:

(i) the expression of ^{a GST polypeptide subunit} ~~the polypeptide according to claim 5 or 6 and, if a further polynucleotide sequence as defined in claim 16 is present, optionally the expression of a further GST subunit encoded by a further polynucleotide, and~~

(ii) the association of the GST subunit polypeptide ~~according to claim 5 or 6 with another GST subunit polypeptide to form a dimeric protein according to claim 7; and~~

(b) recovering the dimeric protein so formed.

19. A process according to claim 17 ~~or 18~~ wherein the cell is a prokaryotic cell or a plant cell.

20. A method of obtaining a transgenic plant cell comprising:

(a) transforming a plant cell with an expression vector according to claim 11 to give a transgenic plant cell,

and optionally,

(a') transforming the cell with one or more further polynucleotide sequences coding for a GST subunit, operably linked to regulatory elements that allow expression of the subunit in the cell.

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21. A method of obtaining a first-generation transgenic plant comprising:
- (b) regenerating a transgenic plant cell transformed with a vector according to claim 11 to give a transgenic plant.
22. A method of obtaining a transgenic plant seed comprising:
- (c) obtaining a transgenic seed from a transgenic plant obtainable by step (b) of claim 21.
23. A method of obtaining a transgenic progeny plant comprising obtaining a second-generation transgenic progeny plant from a first-generation transgenic plant obtainable by a method according to claim 21, and optionally obtaining transgenic plants of one or more further generations from the second-generation progeny plant thus obtained.
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24. A method according to claim 23 comprising:
- (c) obtaining a transgenic seed from a first-generation transgenic plant obtainable by the method according to claim 21, then obtaining a second-generation transgenic progeny plant from the transgenic seed;
- and/or
- (d) propagating clonally a first-generation transgenic plant obtainable by the method according to claim 21 to give a second-generation progeny plant;
- and/or
- (e) crossing a first-generation transgenic plant obtainable by a method according to claim 21 with another plant to give a second-generation

progeny plant;

and optionally;

(f) obtaining transgenic progeny plants of one or more further generations from the second-generation progeny plant thus obtained.

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25. A transgenic plant cell, first-generation plant, plant seed or progeny plant obtainable by a method according to ^{Cl:m 20} ~~any one of claims 20 to 24~~.

26. A transgenic plant or plant seed comprising plant cells according to claim 13 ~~or 15~~.

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27. A transgenic plant cell callus comprising plant cells according to claim 13 ~~or 15, or obtainable from a transgenic plant cell, first-generation plant, plant seed or progeny plant according to claim 25~~.

28. Use of a polynucleotide according to any one of claims 1 to 4 as a selectable marker for detecting transformation of a plant cell.

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29. A nucleic acid construct comprising:

^{Claim 1}
(a) a polynucleotide according to ~~any one of claims 1 to 4~~ operably linked to regulatory elements that allow expression of the coding sequence in a plant cell; and

(b) a site into which a further polynucleotide comprising a coding sequence can be inserted.

30. A nucleic acid construct according to claim 29 wherein site (b) is bounded by regulatory elements that allow expression of a coding sequence inserted

at the site in a plant cell.

31. A vector comprising a construct according to claim 29.

32. A method of transforming a plant cell or of obtaining a plant cell culture or transgenic plant comprising:

(a) providing an untransformed plant cell which is susceptible to a herbicide whose herbicidal activity is reduced by a dimeric protein comprising two GST subunits according to claim 7;

(b) transforming the plant cell with a vector according to claim 29 ~~or 30~~;

(c) cultivating the transformed cell under conditions that allow the expression of the polynucleotide encoding a GST subunit ~~(a) in the construct according to claim 29 or 30~~; and/or

(c') regenerating the cell to give a cell culture or plant such that the polynucleotide ~~(a) in the construct according to claim 29 or 30~~ is expressed; and

(d) contacting the cell, cell culture or plant with the herbicide whose herbicidal activity is reduced by the dimeric protein ~~according to claim 7~~, and to which the untransformed plant cell was susceptible; and

(e) selecting cells, cell cultures or plants that are less susceptible to the herbicide than are corresponding untransformed cells, cell cultures or plants.

33. Use of a dimeric protein according to claim 7 in a method of identifying compounds capable of metabolism by a GST.

34. A method of identifying compounds capable of being metabolised by a glutathione transferase comprising:
- (a) contacting a candidate compound suspected of being capable of being metabolised by glutathione transferase with glutathione (GSH) in the presence of a dimeric protein according to claim 7; and
 - (b) determining whether or not metabolism of the candidate compound takes place.
35. A method according to claim 34 wherein metabolism of the compound is detected by determining whether or not it is conjugated to glutathione by the dimeric protein ~~according to claim 7~~.
36. A kit for detecting compounds capable of being metabolised by a GST comprising:
- (a) reduced glutathione, hydroxymethylglutathione or homoglutathione;
 - and
 - (b) a dimeric protein according to claim 7.
37. An antibody which specifically recognises a polypeptide according to claim 5 ~~or 6~~ or a dimeric protein ~~according to claim 7~~.
Compr: sing said polypeptide
38. A nucleic acid probe which selectively hybridises to the sequence of SEQ ID No. 1, 3, 5, 7, 9, 11, 13, 15 or 17.
39. A method of identifying compounds that induce GST expression in

graminaceous plants comprising:

(a) contacting a graminaceous plant, or a cell or cell culture thereof, with a candidate compound suspected of being capable of inducing GST expression; and

(b) determining the level of GST expression in the plant, cell or cell culture.

40. A method according to claim 39 wherein, in step (b), the level of expression is determined by: (i) determining the level of GST protein present by using an antibody ~~according to claim 35~~; or (ii) determining the level of GST mRNA present using a probe ~~according to claim 37~~.

41. A kit for identifying compounds that induce GST expression in plants ~~by a method as defined in claim 37 or 38~~, which kit comprises an antibody as defined in claim 36.

42. A method of determining the GST level in a sample of seed or flour comprising:

(i) determining the level of GST protein present by using an antibody according to claim 35; or

~~(ii) determining the level of GST mRNA present using a probe according to claim 37.~~

43. A method of controlling the growth of weeds at a locus where a transgenic plant ~~according to any one of claims 25 to 27 is being cultivated~~, which method comprises applying to the locus a herbicide whose herbicidal properties are reduced by a dimeric protein according to claim 7.

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- Claim 34
44. A compound identified by a method according to ~~any one of claims 34, 35, 39 or 40~~
45. A polynucleotide according to claim 1 substantially as hereinbefore described with reference to any one of the preceding Examples.
46. A polypeptide according to claim 5 substantially as hereinbefore described with reference to any one of the preceding Examples.
47. A dimeric protein according to claim 7 substantially as hereinbefore described with reference to any one of the preceding Examples.
48. A chimeric gene according to claim 8 substantially as hereinbefore described with reference to any one of the preceding Examples.
49. A vector according to claim 10 substantially as hereinbefore described with reference to any one of the preceding Examples.
50. A cell according to claim 12 substantially as hereinbefore described with reference to any one of the preceding Examples.
51. A process according to claim 17 or 18 substantially as hereinbefore described with reference to any one of the preceding Examples.
52. A method according to claims 20, 21, 22 or 23 substantially as hereinbefore described with reference to any one of the preceding Examples.
53. A transgenic plant cell, first-generation plant, plant seed or progeny plant, plant or plant seed, or plant cell callus according to any one of claims 25 to 27 substantially as hereinbefore described with reference to any one of the preceding Examples.

54. Use according to claim 28 substantially as hereinbefore described with reference to any one of the preceding Examples.
55. A nucleic acid construct according to claim 29 substantially as hereinbefore described with reference to any one of the preceding Examples.
56. A vector according to claim 31 substantially as hereinbefore described with reference to any one of the preceding Examples.
57. A method according to claim 32 substantially as hereinbefore described with reference to any one of the preceding Examples.
58. Use according to claim 33 substantially as hereinbefore described with reference to any one of the preceding Examples.
59. A method according to claim 34 substantially as hereinbefore described with reference to any one of the preceding Examples.
60. An antibody according to claim 37 substantially as hereinbefore described with reference to any one of the preceding Examples.
61. A nucleic acid probe according to claim 38 substantially as hereinbefore described with reference to any one of the preceding Examples.
62. A method according to claims 39, 42 or 43 substantially as hereinbefore described with reference to any one of the preceding Examples.
63. A compound according to claim 44 substantially as hereinbefore described with reference to any one of the preceding Examples.

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